

Luciferase reporter assay

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 An abbreviated version of this protocol was published in eLIFE in Apr 2021

TAZ-CAMTA1 and YAP-TFE3 alter the TAZ/YAP transcriptome by recruiting the ATAC histone acetyltransferase complex

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Detailed protocol

Luciferase Reporter Assay

1. Plate cells of interest to 60-70% confluence in 3 wells of a 6 well-plate (biological triplicate)
2. Transfect cells of interest with 2µg of pGL3b-8XTTIC firefly luciferase reporter plasmid and 200ng of the pRL-TK *Renilla* plasmid using Lipofectamine with Plus reagent.

The following steps utilize the Dual-Luciferase Reporter Assay from Promega

3. 48 hours after transfection, remove the growth media, wash with 1X PBS, then add 500 µL of 1X passive lysis buffer.
4. Scrape cells, collect the lysate, then incubate on the rocker at room temperature for 15 min.
5. Add 100 µL of Luciferase Assay Reagent II (LAR II) to three wells in a white-walled, clear-bottom, 96 well plate (technical triplicate). LARII should be at room temperature.
6. Transfer up to 20 µL of cell lysate into a well containing 100 µL LARII and mix by pipetting 2-3 times.
7. Record the firefly luciferase activity measurement (program the luminometer to perform a 2-second premeasurement delay, followed by a 10-second measurement period for each reporter assay)
8. Add 100 µL of Stop & Glo Reagent.
9. Record the *Renilla* luciferase activity
10. Normalize the firefly luciferase activity to the *Renilla* luciferase activity

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Tanas, M. R. and Garcia, K. (2022). Luciferase reporter assay. Bio-protocol Preprint. bio-protocol.org/prep1682.
2. Merritt, N., Garcia, K., Rajendran, D., Lin, Z., Zhang, X., Mitchell, K. A., Borchering, N., Fullenkamp, C., Chimenti, M. S., Gingras, A., Harvey, K. F. and Tanas, M. R. (2021). TAZ-CAMTA1 and YAP-TFE3 alter the TAZ/YAP transcriptome by recruiting the ATAC histone acetyltransferase complex. eLIFE. DOI: [10.7554/eLife.62857](https://doi.org/10.7554/eLife.62857)

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